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Luteinizing Hormone Activity Supplementation Enhances Follicle-Stimulating Hormone Efficacy and Improves Ovulation Induction Outcome*

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ABSTRACT

Although FSH is essential to stimulate ovarian folliculogenesis, increasing physiological and clinical evidence suggests that moderate LH stimulation may also be critical for optimal follicle and oocyte development. Conversely, a clinical trend exists toward conducting controlled ovarian hyperstimulation (COH) in a LH-depleted environment, as recently developed gonadotropin preparations are devoid of LH activity, and endogenous LH is suppressed with GnRH analogs in most COH cycles.

To investigate the role of LH activity during COH we supplemented highly purified (HP) FSH with low dose hCG in GnRH agonist-suppressed women. Twenty normoovulatory women were pretreated with a GnRH agonist and after 2 weeks were randomly assigned to receive HP FSH (150 IU/day) alone (group A; 10 patients) or combined with hCG (50 IU/day; group B; 10 patients). The HP FSH dose was increased after 14 days only in cases of inadequate response. Treatment was monitored with pelvic ultrasound and daily hormone determinations. None of the patients of group B and 8 of group A required more than 14 days of treatment and increments of the FSH dose. Folliculogenesis and 17β-estradiol (E_2) secretion progressed more rapidly and evenly in group B. Although preovulatory follicle number and E_2 concentrations were comparable, patients in group B required a shorter stimulation time (12.5 ± 0.6 *vs.* 17.3 ± 0.7 days in group A; $P < 0.0001$) and a lower HP FSH dose (1725 ± 84 *vs.* 2670 ± 164 IU in group A; $P < 0.0001$). Serum levels of LH, E_2, progesterone, and testosterone did not differ between the 2 groups; serum FSH was higher in group A.

We conclude that LH activity promotes folliculogenesis in synergy with FSH in the mid- to late follicular phase and that low dose hCG coadministration optimizes COH by 1) enhancing FSH action, 2) accelerating ovarian follicle development, 3) shortening COH duration, 4) lowering HP FSH requirements, and 5) reducing COH cost. Thus, moderate LH activity in the follicular phase plays a positive physiological and clinical role in folliculogenesis and ovulation induction. (*J Clin Endocrinol Metab* **84:** 2659–2663, 1999)

NUMEROUS infertile patients undergo ovulation induction procedures every year. Up to 2 decades ago ovulation induction was used solely for the treatment of anovulatory infertility; however, the introduction of assisted reproduction technology (ART) has expanded the use of these procedures to eumenorrheic women with the goal of achieving multiple folliculogenesis. Presently used ovulation induction regimens in ART employ moderate or high dosages of exogenous gonadotropins (mostly purified or recombinant FSH) to stimulate multiple folliculogenesis combined with GnRH agonists or antagonists to prevent premature ovulation and follicle luteinization (1).

Thus, because of the suppressive action of GnRH analogs on endogenous gonadotropins and the availability of gonadotropin preparations depleted in LH content, FSH activity predominates in most controlled ovarian hyperstimulation (COH) cycles. FSH is critical for promoting follicle growth and maturation; nevertheless, physiological and clinical evidence suggests that LH may also play important roles in folliculogenesis and ovulation induction (2). Thus, we set up a study to assess the effects of LH activity supplementation in ovulation induction cycles conducted with a fixed regimen of highly purified (HP) FSH in GnRH agonist-suppressed patients. As recombinant human (r-h) LH is not currently clinically available, we elected to employ low dose hCG to simulate LH activity. The results of this study suggest that LH activity synergizes with FSH to enhance follicular and estrogen stimulation during ovulation induction. These findings can be applied to achieve shorter, less expensive, and potentially safer ovarian stimulation regimens.

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Materials and Methods

Patient population

A total of 20 patients suffering form unexplained or male-related infertility were studied. All subjects had regular menstrual cycles of 26- to 34-day duration, a normal body mass index of 20–25 kg/m^2, a pelvic ultrasound showing uterus and ovaries of normal size and structure, a hysterosalpingogram and/or laparoscopy demonstrating tubal patency, normal plasma and urinary chemistry and hematological values, and thyroid and reproductive hormones within the normal range. Although ovulation induction had been previously performed in some of the subjects, no patient had received any hormone therapy (including gonadotropins) for at least 3 months preceding the study.

Protocol

The protocol was approved by our institutional review board, and all patients provided informed consent. Patients underwent early follicular phase reproductive hormone determinations and were then randomly assigned to two age- and weight-matched groups. Patients were not

blinded to treatment. Treatment was started in the midluteal phase of a spontaneous menstrual cycle with the administration of a single injection of 3.75 mg depot triptorelin (Decapeptyl 3.75, IPSEN S.p.A., Milan, Italy). Ovulation induction was started 14 days thereafter. Patients of group A received 150 IU, sc, highly purified (HP) FSH (Metrodin HP, Serono Pharma S.p.A., Rome, Italy) daily between 1400–1600 h, and patients of group B received the same dose and schedule of HP FSH plus 50 IU, sc, hCG (Profasi HP, Serono Pharma S.p.A.) daily. In both groups the gonadotropin administration schedule was not changed for 14 days or until at least four ovarian follicles of more than 14 mm diameter and 17β-estradiol (E_2) levels of 800-1500 pg/mL were detected (final maturation parameters). If these parameters were not achieved by the 14th day of treatment, increments in the HP FSH dose alone were allowed with the following schedule: 225 IU daily on days 15–17 and 300 IU daily on days 18–20. Treatment was to be discontinued on day 21 if the final maturation parameters had not been achieved. At the obtainment of the final maturation parameters, 10,000 IU hCG were administered to trigger ovulation, and homologous intrauterine insemination with a sperm swim-up procedure was performed 36 h thereafter. The luteal phase was supported with 90 mg daily of intravaginal progesterone (P) gel (Crinone, Wyeth Lederle S.p.A., Aprilia, Italy) administered form the 3rd to the 14th day after the preovulatory hCG dose.

Monitoring

Treatment monitoring was conducted throughout menotropin administration. Each day one blood sample was drawn between 0800–0900 h in a standard manner, and two serum aliquots were obtained: E_2 was measured daily in one of the serum aliquots for clinical monitoring, and the second aliquot was stored at −20 C for later measurements of LH, FSH, E_2, P, testosterone (T), and hCG. Transvaginal pelvic ultrasound was performed until preovulatory hCG administration on menotropin treatment days 0, 6, 8, 10, 12, 14, 16, 18, and 20.

Hormone assays

LH, FSH, E_2, P, T, and hCG were measured with chemiluminescence assays (Chiron Corp. Diagnostics ACS 180, Milan, Italy). The minimal detectable dose (MDD) of LH was 0.1 IU/L; intra- and interassay coefficients of variation (CVs) were 5.3% and 6.0%, respectively. The MDD of FSH was 0.3 IU/L; intra- and interassay CVs were 4.1% and 4.9%, respectively. The MDD of E_2 was 10 pg/mL; intra- and interassay CVs were 3.3% and 7.0%, respectively. The MDD of P was 0.1 ng/mL; intra- and interassay CVs were 6.2% and 8.0%, respectively. The MDD of T was 0.1 ng/mL; intra- and interassay CVs were 5.8% and 8.0%, respectively. The MDD of hCG in this β-specific assay was 0.1 IU/L; intra- and interassay CVs were 4.5% and 7.0%, respectively.

Statistical evaluation

Data were expressed as the mean ± SE. Serum hormone levels during treatment were calculated in each cycle as the area under the curve (AUC). Between-group differences of continuous variables were assessed with Student's *t* test or the Mann-Whitney rank sum test, as appropriate.

Results

The patient characteristics of the two treatment groups are shown in Table 1. No significant between-group differences existed in age, height, weight, body mass index, or baseline hormone levels. All patients completed the treatment schedule and appeared to ovulate. Three conceptions were achieved, two in group A and one in group B; however, both pregnancies in group A underwent early miscarriage, whereas the pregnancy of group B is ongoing. No multiple gestations or ovarian hyperstimulation syndrome cases occurred.

The clinical and endocrine results of treatment are shown in Table 2. The final outcome of treatment did not differ, as preovulatory E_2 levels and the number of small (<10 mm), medium (10–14 mm), and large (>14 mm) ovarian follicles were comparable in groups A and B. Although the duration of treatment in group B ranged between 8–14 days, and none of these patients had to undergo increment of the HP FSH dose, treatment in group A lasted 13–19 days, and 8 of 10 of these patients underwent 1 or 2 HP FSH dose increments. Therefore, both the duration of gonadotropin administration and the dose of HP FSH employed were significantly increased in group A (Table 2); these parameters were 38% and 55% higher in group A than in group B, respectively.

The AUC of LH, E_2, P, and T during treatment did not significantly differ between the two treatment groups. Conversely, the AUC of FSH was significantly more elevated in group A (Table 2); hCG was detectable only in group B. The analysis of hormone profiles (Fig. 1) indicated that serum LH levels progressively declined in both groups during gonadotropin administration from day 0 (group A, 1.4 ± 0.1 IU/L; group B, 1.7 ± 0.1 IU/L) to day 8 [group A, 0.7 ± 0.1 IU/L ($P < 0.0001$); group B, 1.2 ± 0.1 IU/L ($P < 0.05$)]. Conversely, serum FSH rapidly increased, plateaued by treatment day 4, and remained elevated throughout treatment in both groups (Fig. 1). Serum E_2 also increased in both groups, but the rise was more rapid in group B as mean serum E_2 levels above 700 pg/mL were achieved by treatment day 10 in this group and only by day 16 in group A. The pattern of P and T secretion did not seem to be affected by gonadotropin treat-

TABLE 1. Baseline characteristics of the two groups of patients

	Group A	Group B	*P*
Age (yr)	32 ± 1	33 ± 1	NS
Ht (cm)	163 ± 2	166 ± 1	NS
Wt (kg)	60 ± 2	61 ± 2	NS
BMI (kg/m^2)	22.4 ± 0.5	22.2 ± 0.5	NS
LH (IU/L)	3.1 ± 0.3	3.1 ± 0.3	NS
FSH (IU/L)	6.7 ± 0.3	6.4 ± 0.4	NS
PRL (ng/mL)	13.7 ± 1.5	14.1 ± 1.2	NS
E_2 (pg/mL)	70 ± 5	65 ± 6	NS
P (ng/mL)	0.4 ± 0.1	0.5 ± 0.1	NS
T (ng/mL)	0.5 ± 0.1	0.5 ± 0.1	NS

TABLE 2. Clinical and endocrine response parameters in the two groups of patients

	Group A	Group B	*P*
Days of gonadotropin administration (range in *parentheses*)	17.3 ± 0.7 (13–19)	12.5 ± 0.6 (8–14)	<0.0001
HP FSH dose (IU)	2,670 ± 164	1,725 ± 84	<0.0001
Preovulatory E_2 (pg/mL)	977 ± 27	1,171 ± 151	NS
Preovulatory follicles			
<10 mm	3.2 ± 0.6	3.2 ± 0.4	NS
10–14 mm	3.8 ± 0.5	3.8 ± 0.4	NS
>14 mm	7.9 ± 0.8	8.7 ± 0.7	NS
Follicular phase hormone levels			
LH (IU/L·day)	13 ± 1	14 ± 1	NS
FSH (IU/L·day)	146 ± 11	112 ± 8	<0.03
E_2 (pg/mL·day)	3,529 ± 276	3,163 ± 240	NS
P (ng/mL·day)	6.4 ± 1.0	5.6 ± 0.7	NS
T (ng/mL·day)	4.3 ± 0.5	4.0 ± 0.4	NS
hCG (IU/L·day)	<0.1	16.2 ± 3.2	<0.0001

Follicular phase hormone levels were calculated as the area under the curve.

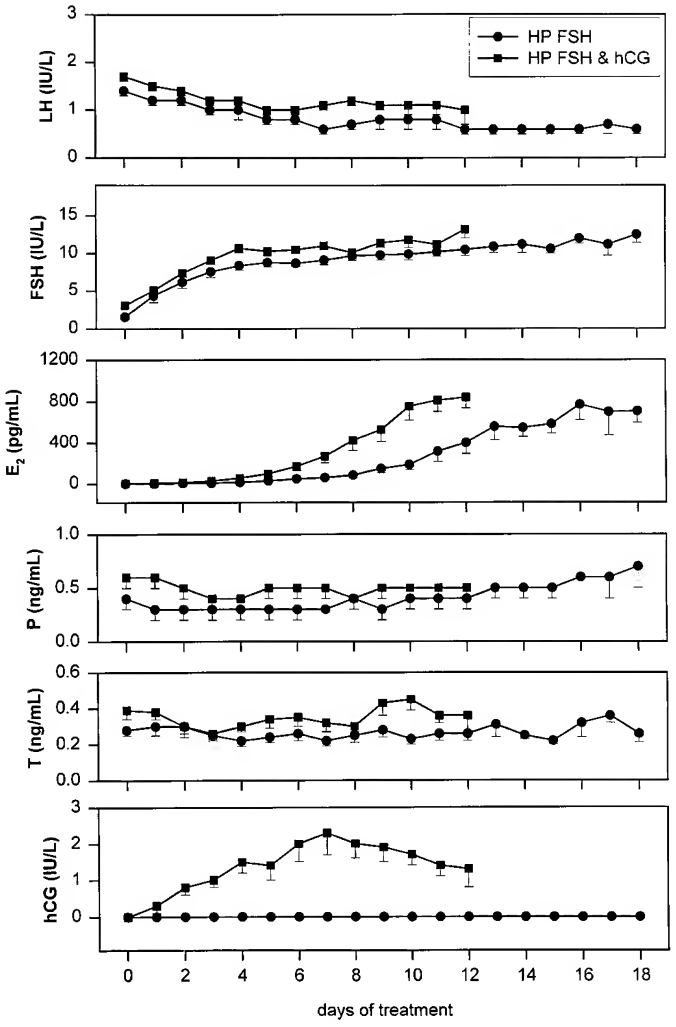

FIG. 1. Gonadotropin and gonadal steroid serum levels (mean ± SE) during HP FSH treatment.

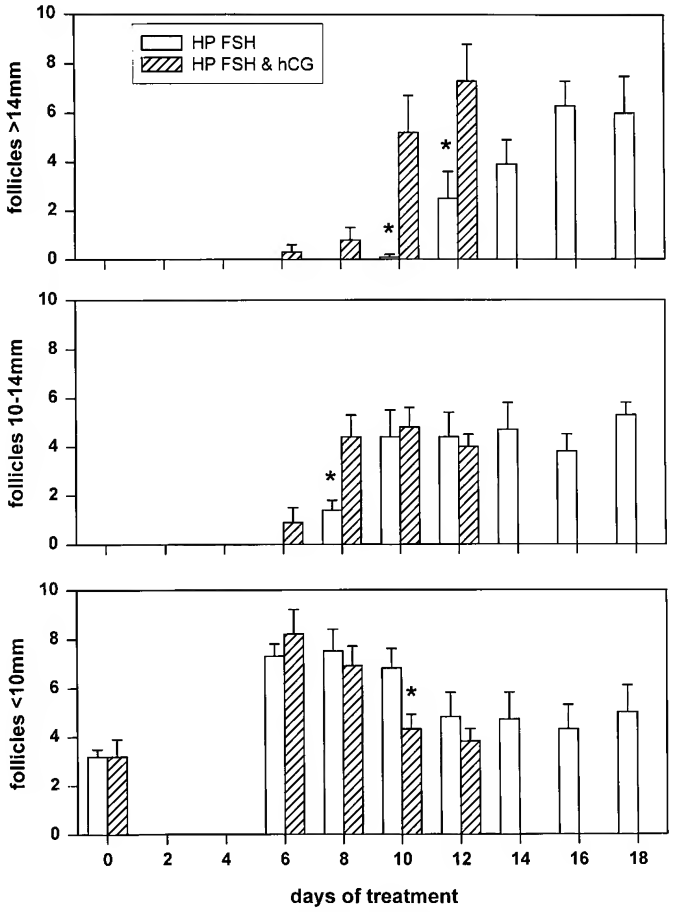

FIG. 2. Number (mean ± SE) of small (<10 mm diameter), medium (10–14 mm), and large ovarian follicles (>14 mm) measured during HP FSH treatment. On treatment day 0, only small follicles were detected, whereas pelvic ultrasound was not performed on days 1–5. *, $P < 0.05$ or less (see *Results*).

ment, as no significant difference in the concentrations of these hormones was found between the treatment groups (Table 2). Finally, serum hCG remained undetectable throughout treatment in group A, whereas in group B it increased progressively until day 7 and remained stable at levels between 1–3 IU/L, thereafter.

The pattern of ovarian follicle development is shown in Fig. 2. Although the number of small follicles (<10 mm diameter) rapidly increased in both groups in the initial days of treatment, the emergence of intermediate (10–14 mm) and large follicles (>14 mm) was delayed by 2–4 days in group A compared to that in group B. The number of large follicles was greater in group B on both day 10 (5.2 ± 1.5 *vs.* 0.1 ± 0.1; $P < 0.005$) and day 12 (7.3 ± 1.5 *vs.* 2.5 ± 1.1; $P < 0.05$). The number of medium size follicles was greater in group B on day 8 (4.4 ± 0.9 *vs.* 1.4 ± 0.4; $P < 0.05$). Finally, the number of small follicles was greater in group A on day 10 (6.8 ± 0.8 *vs.* 4.3 ± 0.6; $P < 0.05$).

Discussion

The pituitary gonadotropin FSH is unquestionably the key regulator of ovarian follicle development (3), and granulosa cells express FSH receptors starting during prenatal life (4). The role of LH in ovarian physiology is more controversial. Thecal cells express LH receptors during fetal development, and LH stimulation provides the androgen substrate required for estrogen production by granulosa cells (5, 6). However, LH receptors are also present on granulosa cells in the late stages of follicle development, so that these cells become responsive to LH stimulation in the mid- to late follicular phase of the menstrual cycle (5, 7). Although the appearance of LH receptors had been considered critical only for the enzymatic, endocrine, and morphological changes in granulosa cells related to ovulation and follicle luteinization, the results of this study suggest that LH activity directly stimulates follicle growth and maturation, starting in the midportion of the follicular phase.

For the first time we unequivocally demonstrated that LH activity supplementation enhances FSH activity and optimizes FSH ovulation induction in women undergoing COH for ART procedures. The original feature of our protocol was the administration of a fixed regimen of HP FSH in GnRH agonist-suppressed women, with or without low dose hCG supplementation to provide LH activity stimulation. Our choice of submitting all patients to an invariable HP FSH

dose in the initial 14 days of treatment was finalized to best unravel the specific effects of gonadotropins on folliculogenesis and gonadal steroid secretion. Most previous studies in which different gonadotropin preparations were compared allowed gonadotropin dose adjustments dependent upon individual patient response (8–10). This approach renders assessment of differences in gonadotropin response more difficult because, as shown in this study, increasing the intensity of FSH stimulation will eventually lead to acceptable degrees of ovarian stimulation in most patients. We also elected to submit all patients to a long regimen of depot GnRH agonist suppression that resulted in a profound reduction of endogenous LH so that the effects of exogenously administered LH activity could be best assessed. This type of regimen is currently clinically employed in a large number of ART cycles and, as shown in our study, will reduce LH to levels considered incompatible with optimal ovarian stimulation and ART results (11–13). Finally, we elected to provide LH activity by administering hCG at a low dose (50 IU/day), as r-hLH is not yet approved for clinical use; nevertheless, it is well known that exogenous hCG has been previously used in commercial human menopausal gonadotropin (hMG) preparations to correct imbalances in the LH/FSH ratio (14).

A recent study conducted in patients with profound hypogonadotropic hypogonadism (15) demonstrated that r-hLH administration is required to achieve proper follicle development and estrogen secretion; in that study FSH-only stimulation in patients who were profoundly deprived of endogenous LH activity resulted in reduced preovulatory follicle number and lower ovulatory and pregnancy rates. Our study extends the concept of the value of LH stimulation to a far more common condition, *i.e.* normoovulatory women in whom LH is pharmacologically reduced with a GnRH agonist for COH. In the present study we found that patients treated with HP FSH alone or with low dose hCG supplementation eventually achieved comparable levels of preovulatory E_2 and large ovarian follicles. This confirms the finding of most previous studies (2) and clinical experience that HP FSH can be effectively used for most patients undergoing COH. Nevertheless, when HP FSH was supplemented with LH activity in the form of low dose hCG, ovarian stimulation was more efficient. Although none of the patients in group B had to be treated for more than 14 days or had to receive an increased HP FSH dose, 80% of the patients in group A required a lengthened and more intense gonadotropin stimulation. The mean duration of treatment and the mean HP FSH dose were increased in group A by 38% and 55%, respectively. These findings demonstrate that comparable folliculogenesis can be obtained more rapidly and with the use of markedly less HP FSH when LH and FSH activity is combined for ovarian stimulation. Although hMG-associated reduction of the menotropin dose was occasionally reported (16), our results contradict most previous studies in which FSH alone and hMG (which contains both LH and FSH) were compared (2). However, previous investigations in this area were not conducted with a fixed regimen of gonadotropin stimulation and/or did not analyze GnRH agonist-suppressed patients (10).

The serum FSH profile was similar in the two treatment groups during the initial 12 days of treatment; however, FSH AUC concentrations were increased in group A, reflecting greater dose and duration of HP FSH administration. Conversely, serum LH levels progressively declined across the follicular phase in both groups and reached mean levels around or below 1 IU/L within a few days. As previously reported (11–13), these LH levels may be inadequate for proper follicle stimulation. However, at the same time serum hCG levels in group B rapidly reached mean levels between 2–3 IU/L, thus compensating for lost endogenous LH activity. Stable serum hCG levels after 7 days of treatment indicate that a hCG steady state was rapidly achieved despite a long hCG half-life. Serum P and T levels were not significantly increased in group B, suggesting that the low dose hCG regimen we chose (50 IU daily) did not excessively stimulate thecal cell function or cause follicle luteinization.

LH activity supplementation accelerated the process of folliculogenesis as attested by the finding that peak preovulatory E_2 levels and medium and large follicles were achieved an average of 4 days earlier in group B. Analysis of pelvic ultrasound results suggests variable effects of LH and FSH on follicles of different sizes. Small (<10 mm) follicles rapidly increase in number at the outset of treatment in both groups, confirming that FSH alone affects growth dynamics at this stage of follicular development. However, the development of medium (10–14 mm) and large (>14 mm) follicles was selectively enhanced in patients receiving low dose hCG supplementation, suggesting that expression of LH receptors may provide an additional source of follicular support at this stage of follicle development. This concept is supported by the recent findings of Sullivan *et al.* (12), who showed that LH alone was capable of sustaining E_2 production once a follicular size greater than 14 mm in diameter was achieved. In a previous study by Thompson *et al.* (17), 50–75 IU hCG daily were used in combination with 150–225 IU FSH daily in GnRH antagonist-suppressed women, but failed to affect follicle development or E_2 concentrations. However, in that study both gonadotropins and GnRH antagonist administration were started during the late follicular phase of a spontaneous menstrual cycle, and this short period of endogenous LH deprivation may have been insufficient to affect the final stages of a single dominant follicle maturation. Conversely, a recent large multicenter study (18) clearly showed that high doses of the GnRH antagonist Ganirelix combined with r-hFSH administration were associated with reduced preovulatory LH levels to an extent similar to what we encountered in our protocol; in these patients, E_2 levels and pregnancy rates at ART were reduced, whereas miscarriage rates were increased. Although our series is too limited to draw any conclusion in terms of viability of pregnancy, it is interesting to note that both patients who conceived in group A also experienced early spontaneous abortion.

In summary, the results of our study indicate that supplementation of HP FSH treatment with low dose hCG in LH-deprived women allows to optimize ovulation induction through shorter treatment and lower HP FSH dose. These findings confirm and extend the concept that LH plays a pivotal role in the final stages of follicular maturation, notwithstanding that LH deprivation can be partly compensated by more intense or prolonged FSH stimulation. These

results also suggest that moderate LH stimulation can be used to reduce ovulation induction costs by decreasing exogenous FSH dose requirements and by shortening treatment duration and monitoring. Furthermore, as the occurrence of ovarian hyperstimulation syndrome is closely related to the degree of exogenous FSH stimulation and the presence of small ovarian follicles, it is conceivable that drug regimens that combine LH and FSH activities may yield a lower incidence of this dreaded complication of ovulation induction.

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